LARVAL TREMATODES FROM AUSTRALIAN TERRESTRIAL AND FRESHWATER MOLLUSCS

PART III. LEUCOCHLORIDIUM AUSTRALIENSE, N. Sp.

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Leucochloridium australiense, n. sp.

Specimens of the terrestrial gastropod, *Succinea australis* (Ferussac), collected by Mr. F. Jaensch at Elwomple, near Tailem Bend, on June 24, 1937, were examined a month later, and in one a large pulsating sac which contracted rhythmically for some hours, was found alongside the liver. Upon dissection the main part of the sporocyst to which the sac was attached was uncovered. It consisted of a central portion from which arose about six juvenile pulsating structures and numerous knob-like, club-like and finger-like projections, many of the latter being of considerable length and ramifying for some distance through the tissues of the snail.

The main part of the sac (fig. 3) was white with distally-situated coloured bands, separated by slight constrictions. The most proximal of these bands was an incomplete brown ring, the second and third were complete brown bands, the fourth a pale shade of green, the fifth a complete brown ring, the sixth an incomplete brown band, and the tip of the sac was brown. Each of these rings was separated by a colourless band, in the centre of which (except between the fifth, sixth, and tip of the sac) was an opaque white line at the point of constriction.

The Cercariaeum

About twenty fully developed cercariaea were found in the large pulsating sac. Each was enclosed within a thick gelatinous sheath (fig. 2) interrupted at both the anterior and ventral suckers. In the outer part of the sheath faint concentric and radial lines were seen.

Each cercariaeum (figs. 1, 2, 4) was capable of much contraction and expansion, and a typical one was 616 μ long and 347 μ broad when contracted, and 308 μ broad when extended. The almost circular anterior sucker, which was surrounded by an elevated margin more pronounced dorsally, measured 193 μ across, and the mouth was subterminal on its ventral surface just below the tip. A powerful, almost circular, pharynx, 69 μ long, was present, and from its dorsal aspect arose a very short oesophagus. This divided almost immediately to form the two intestinal caeca which arched upwards and outwards, and then passed backwards on either side of the ventral sucker to the level of the genital pore.

The ventral sucker lying in the anterior part of the second half of the body was 154 μ in diameter. The anterior sucker, pharynx and ventral sucker (figs. 11, 12, 20) were formed of radiating muscle fibres associated with large vacuolate myoblasts with prominent nuclei. Circular sphincter-like and longitudinal to oblique muscle fibres were present just beneath the cuticle, while internally to the radial fibres they were most strongly developed. At the junction of the anterior sucker and pharynx the circular fibres were much more numerous, and at the rim of the two suckers were grouped to form sphincters.

The intestine was lined by cuticle and cuboidal cells, and beyond the latter a few circular muscle fibres.

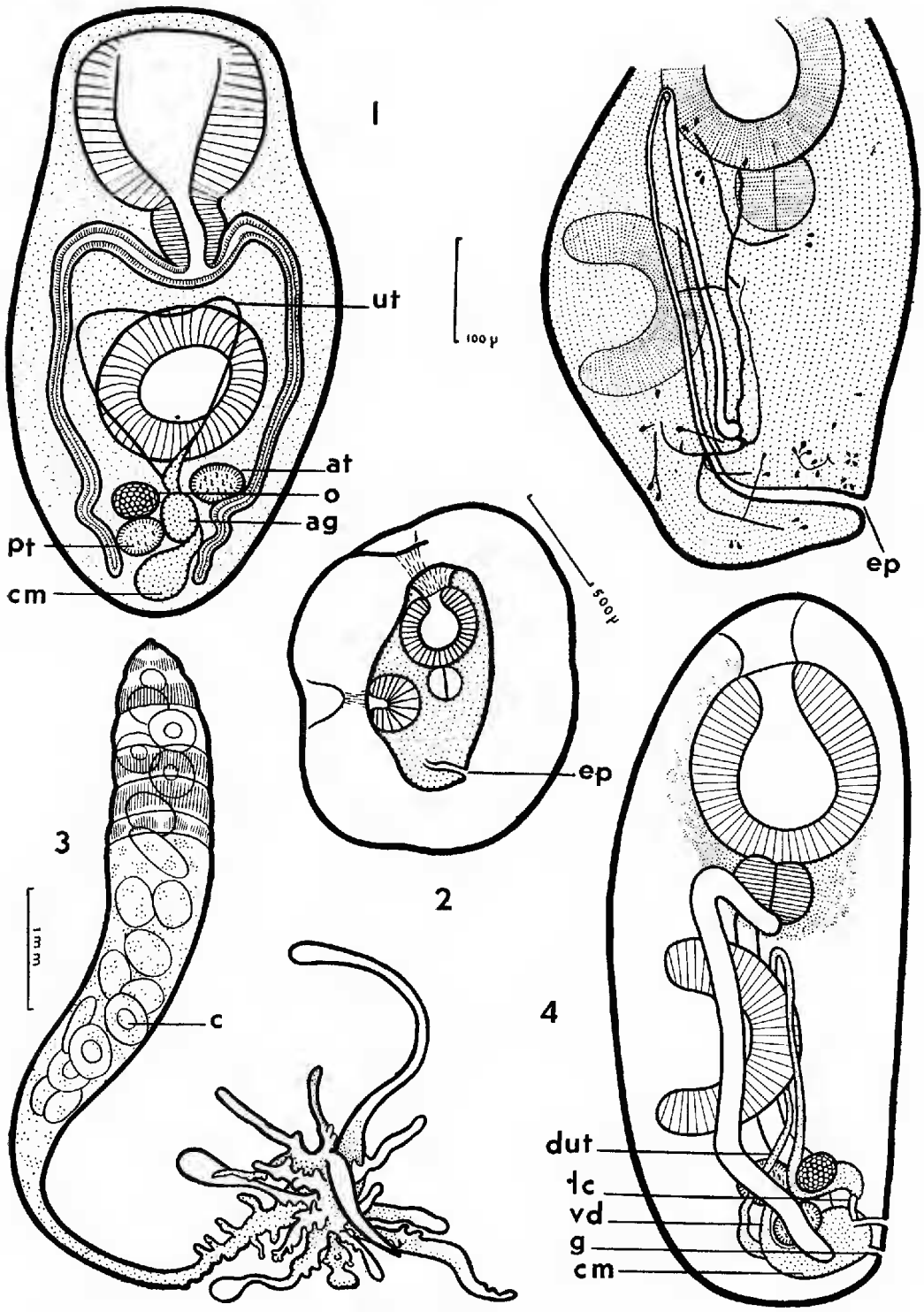
The general body surface was covered with a thick cuticle, but no trace of cilia was seen, though Magath (1920) and Zeller (1874) reported their presence in other species. Underlying the cuticle were circular, longitudinal and oblique muscle fibres supported by large connective-tissue cells (fig. 15). Scattered muscle fibres were seen throughout the body.

The nervous system (fig. 11) was typical and consisted of two lateral brain masses, one on either side of the anterior sucker and pharynx and connected dorsally by commissures. A large ventral nerve could be traced backwards on each side, and the root of each narrow dorsal nerve was seen. The anterior sucker was supplied by nerves from the brain.

Sense cells (figs. 13, 14, 20) were present on the surface of both suckers and an occasional minute one was seen in the cuticle of the body surface in the level of the pharynx. They were specially prevalent at the edge of the mouth and varied considerably in size, the largest being at the base of the anterior sucker immediately before its junction with the pharynx. They were either stalked or sessile, and consisted of a central clear parenchymatous part (fig. 14), in which was embedded the nerve fibrils, the whole surrounded by a substance having the consistency and colour of the cuticle. Dorsally and laterally from the pharynx a small number of cells similar to these in size and structure and staining properties were seen embedded in the parenchyma, but it is difficult to account for their function in such a situation.

Reproductive System

The two oval testes were diagonally placed, the anterior on the right-hand side (as viewed through a compound microscope) a little distance behind the ventral sucker; the posterior slightly dorsal to it but on the left-hand side. Above the posterior testis and slightly dorsal and median to it was the oval ovary, the three gonads thus forming a triangle (figs. 1, 4). In some specimens the ovary was found lying slightly in front of the anterior margin of the anterior testis. From the posterior testis the vas efferens (fig. 17) passed obliquely upwards ventral to the ovary and was joined by the shorter duct from the anterior testis. From this point the vas deferens (figs. 8, 16) travelled backwards, then turned sharply dorsally and passed through an undifferentiated cell mass to open at the



Figs. 1-5

Fig. 1, cercariaeum, dorsal view; 2, cercariaeum in sheath; 3, sporocyst and pulsating sac; 4, cercariaeum, lateral view; 5, excretory system.

Figs. 1 and 4 drawn to scale beside fig. 1; figs. 2 and 3 to scale indicated beside each.

gonopore on the dorsal side of the animal (figs. 6, 19, 20) a short distance from the posterior end. No seminal vesicle or true cirrus could be seen. The undifferentiated cell mass (figs. 1, 4, 6, 19, 20) near the gonopore was large and surrounded the end parts of the uterus and of the vas deferens, and thus could not be described at this stage as a cirrus sac. It gradually tapered ventrally and anteriorly away from the gonopore and then became separated into two parts, one of which surrounded the vas deferens and the other the uterus.

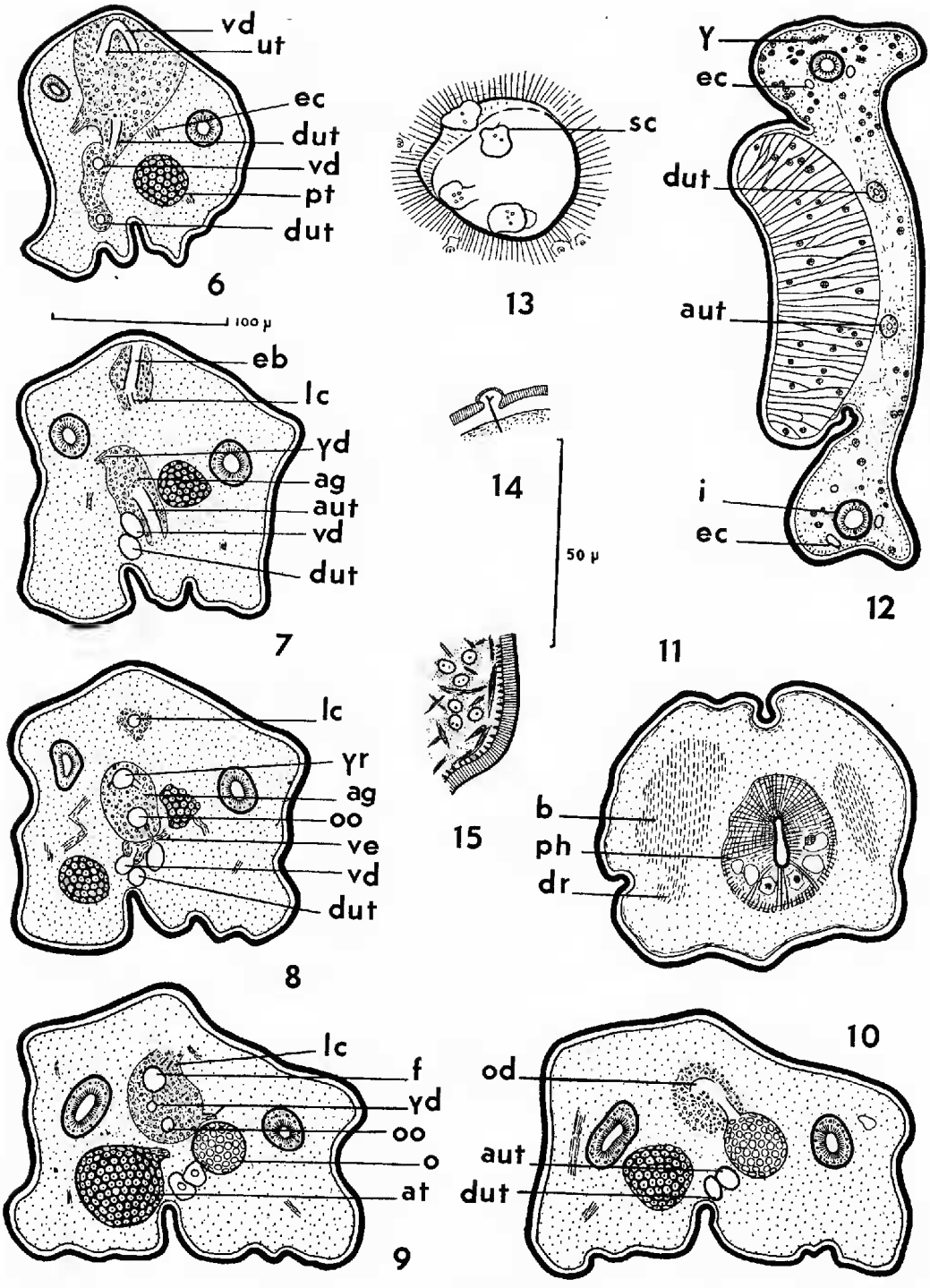
The short oviduct (fig. 10) travelled towards the mid-line, where it was joined by Laurer's canal (figs. 7, 8, 9, 19, 20), which passed posteriorly to enter the excretory canal just before the latter reached the excretory pore. Magath (1920, 109, 111) reported that a similar condition was present in *L. problematicum*, and was described by Looss (1899) for *L. insigne*. Near the junction of Laurer's canal with the oviduct was a slightly swollen part of the canal, probably the anlage of the fecundarium. The oviduct, after its junction with Laurer's canal, turned ventrally and was then joined by a very short vitelline duct (figs. 8, 9) which passed backwards to become widened into a small reservoir receiving the two yolk ducts. The latter ducts curved ventrally and anteriorly to the slightly developed yolk glands lying laterally from the intestinal caeca. Surrounding the oviduct, yolk reservoir and fecundarium was a large mass of undifferentiated tissue, the albumen gland (figs. 1, 4, 7-10).

After its junction with the yolk duct, the oviduct continued to the mid-ventral line, where it passed forwards into the ascending uterus (figs. 8, 9). This travelled upwards and outwards on the inner side of the anterior testis, formed a loop around the dorsal portion of the ventral sucker (figs. 1, 4) and descended on the other side, passing gradually towards the median line until, just behind the sucker, it lay alongside its ascending branch. It then proceeded posteriorly to the level of the gonopore, turned sharply dorsally, became associated with the tissue of the undifferentiated cell mass, and joined the vas deferens immediately before the latter opened at the gonopore.

Excretory System

The excretory pore (fig. 5) was on the dorsal surface immediately above the genital opening, and led into a small rounded excretory bladder (fig. 7). The latter received Laurer's canal dorsally (figs. 4, 7, 20), while laterally it gave rise to two main collecting tubes (fig. 6) which passed upwards, external to the intestinal caeca, to well beyond the base of the anterior sucker. Here these canals bent backwards until they reached the level of the posterior region of the sucker, where they became dilated just before giving rise each to an anterior and a posterior collecting tubule.

The anterior tubule passed forwards and, in the region of the ventral sucker, gave rise to a dorsal branch and a short ventral branch which appeared to join the main ascending tube; the main stem then continued to the level of the pharynx, where it divided into three branches; one of these passed dorsally



Figs. 6-15

Figs. 6-12, Tr. sections of cercariaeum; 7, 8, are consecutive sections; 13, sense cells at base of anterior sucker; 14, longitudinal section of sense cell; 15, longitudinal section of body wall.

Figs. 6-12 drawn to scale below fig. 6; figs. 13-15, to scale below fig. 14.

below the pharynx, while the second and third travelled forwards, one lateral and the other ventro-lateral to the pharynx.

The short posterior tubule almost immediately gave rise to several accessory branches. The first passed upwards alongside the anterior collecting tubule, the second between the ascending and descending main tubes; the third, fourth and fifth were terminal, the third proceeding anteriorly to end behind the ventral sucker, the median fourth lying between the other two and travelling backwardly towards but below the excretory bladder, and the fifth dorsally towards but above the bladder. The bladder and the proximal ends of the main excretory tubes were lined with cuticle.

The correct number and arrangement of the flame cells and excretory tubules could not be determined owing to the small number of cercariaea available for study, their thickness, and the small size of the flame cells. The figure and descriptions of this system, therefore, give only an approximation of their arrangement.

Relationships

The cercariaeum stage of *Leucochloridium australiense* differs from that of *L. macrostomum* (Rud.) and *L. problematicum* Magath in size, and also in the absence of cilia on the general body surface. It is slightly smaller than *L. macrostomum* which is 0·85 mm. long and 0·45 mm. broad, and much smaller than *L. problematicum* which is 2·2 mm. by 0·85 mm. The anterior sucker, pharynx and ventral sucker also differ considerably and in *L. australiense* are almost circular, measuring, respectively, 193 μ, 69 μ, and 154 μ in diameter. In *L. problematicum* they measure, respectively, 0·4 mm. long and 0·24 mm. wide; 0·17 mm. and 1·15 mm.; and the almost circular ventral sucker 0·34 mm.

Laurer's canal in the Australian species opens into the excretory bladder as in *L. problematicum,* and not on the dorsal surface as in *L. macrostomum;* while the intestine resembles that of the former species.

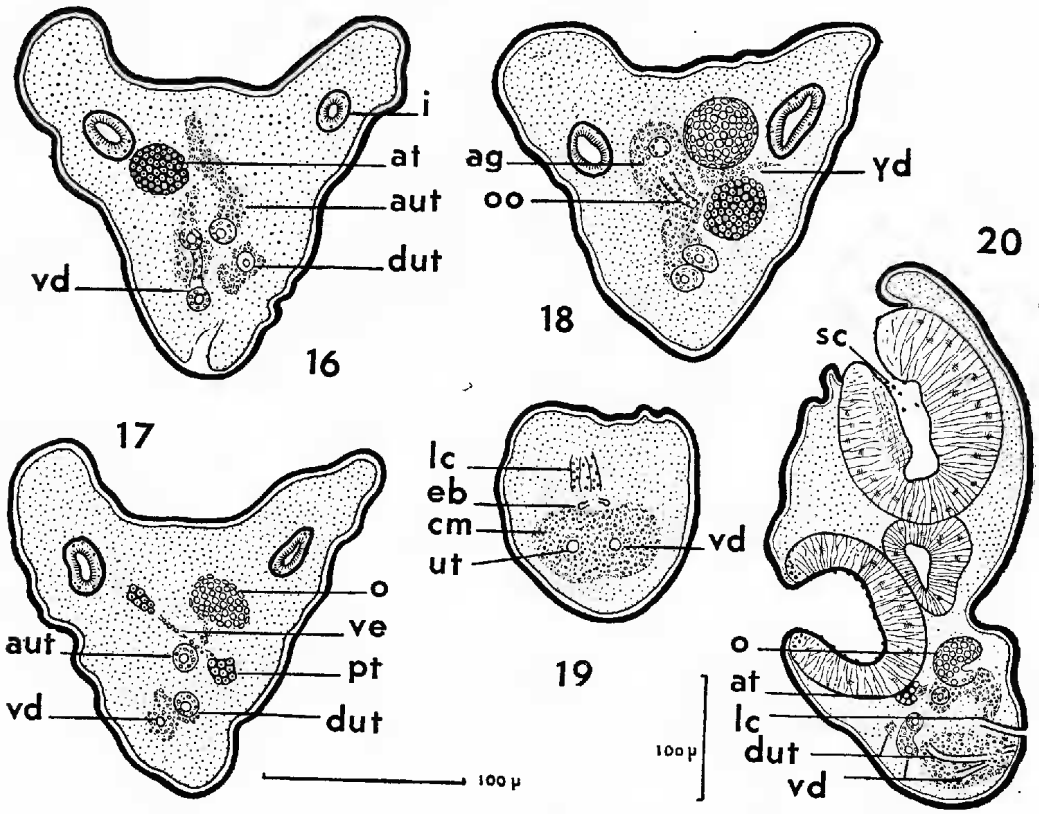
The reproductive system differs in the relationships of the ovary and the two testes from *L. assamense* Sewell (1922), and is similar to that of *L. macrostomum* and *L. problematicum,* except that in our specimens no true cirrus sac is present as the uterus and vas deferens pass together through an undifferentiated cell mass prior to opening at the gonopore. This condition may perhaps become altered in later larval development.

Our species appears to come nearest to *L. problematicum* and *L. insigne.* In the study of species of *Leucochloridium* more attention has been paid to the natural history of the mother sporocysts than to the cercariaeum, with the result that few of the latter have been adequately described. The first of these, *L. macrostomum* (Rud.), was described by Heckert in 1888 under the name of *L. paradoxum,* and an account of its histology, morphology and life history was given.

In 1920 Magath described a new species, *L. problematicum,* from North America, which greatly resembled the marita of *L. insigne* described by Looss in

1899 from European birds. Magath suggested that *L. problematicum* was the parthenita stage of *L. insigne,* although the host of the latter, *Fulica atra,* was stated not to occur in the region from which his material was derived. In view of later data on the host specificity of species of *Leucochloridium,* Magath's suggestion is probably incorrect.

Sewell, in 1922, described the third cercariaeum as *L. assamense,* which does not seem to us to be a typical member of the genus.



Figs. 16-20.
Figs. 16-19, longitudinal horizontal sections of cercariaeum;
20, longitudinal vertical section of cercariaeum.
All drawn to same scale.

Sinitsin (1931, 796) gave a brief summary of investigation on the family Harmostomidae, and included a revised classification of the Harmostominae (thus excluding the Leucochloridiinae) and descriptions of various species. He pointed out that the parthenita stage of the latter is specific and the marita stage indiscriminate in regard to host relationship. But such a statement does not apply to the Leucochloridiinae.

McIntosh (1933) described six new species of *Leucochloridium* (marita stage) and included a key for the differentiation of all known species. This was largely based upon the distribution of the vitellaria, size of fecundarium, etc.;

characters which could not be determined satisfactorily in the larva, and were, therefore, not of much assistance to us in placing our new form. An important deduction from this paper, mentioned by Woodhead (1935), is that species of this genus are specific in their bird hosts.

Wesenberg-Lund (1931) gave a full account of the biology of *L. paradoxum (i.e., macrostomum)* and discussed the papers of Heckert, Magath and Mönnig. He believed that Magath had erected his new species *L. problematicum* on insufficient data, and suggested that the brown sacs described by that investigator in America belonged to the same species as those found by Heckert and Mönnig in Europe. But it seems to us possible that sporocysts, apparently similar, may give rise to different cercariaea. In one of his figures, Wesenberg-Lund (1931, 95, fig. 3) shows a cercariaeum from a brown sac and one from a green sac, and mentioned a slight difference in regard to the sizes of the suckers. His figure indicates the ratio of the anterior to the ventral sucker as 5:4 in the case of the cercariaeum from brown sac, and 1:1 from that from a green sporocyst. In his later figures (Wesenberg-Lund, 1934, pl. xxxii, figs. 7, 8) a slight difference is to be observed in the sucker ratio of the two cercariaea assigned to *L. paradoxum*. This ratio is 4:3 in the cercariaeum in fig. 8, and 10:9 in the cercariaeum in fig. 7, but Wesenberg-Lund does not state from what kind of sac they were obtained.

Lühe (1909, 209, fig. 188) has drawn a lateral view of the cercariaeum of *L. macrostomum*. Woodhead (1935) gave a description of four new *Leucochloridium* sacs, one of which is very like, and may prove to be identical with, that already described by Magath. He remarked upon the specificity of *Leucochloridium* maritae as regards their hosts, implied in McIntosh's paper, but this theory, according to Wesenberg-Lund (1931, 133, 134), is not substantiated on account of the presence of the maritae of *L. macrostomum* and *L. insigne* in a number of different bird hosts. In 1936 he referred to an extraordinary case of multiple infection of *Succinea retusa* with the sporocysts of *Leucochloridium*.

Gower (1936) gave a description of a new sporocyst of *Leucochloridium* from Louisiana and included a camera-lucida drawing of the cercariaeum. This, he stated, differed from *L. problematicum* in the sucker ratio, which was approximately 2:1 in his specimen, but he gave no account of the anatomy.

Yamaguti (1935, 173) described a new marita, *L. sime*, which resembled most closely *L. variae* McIntosh (1932).

Addendum

Since this paper was accepted for publication, Mönnig's (1922) important paper on *Leucochloridium macrostomum* has become available. His account of the female ducts does not agree with their disposition in our material, and we would suggest that he has probably confused the ascending and descending limbs of the uterus in the vicinity of the albumen gland. We were unable to find any connection between the albumen gland and the enlarged part of the descending uterus such as he indicates in his fig. 21.

The sense cells referred to in our paper as occurring in the vicinity of the mouth and pharynx may perhaps be similar to structures indicated in his pl. v, fig. 27, and which he has called "epithelial cells" and "pharyngeal pocket epithelium" respectively. The distribution of colouration of the pulsating sacs differs considerably for the Australian and European forms as figured by him.

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EXPLANATION OF LETTERING

All drawings were made with the aid of the camera-lucida, except fig. 5.

ag, albumen gland; at, anterior testis; aut, aseending limb of uterus; b, brain; c, cercariaeum; cm, undifferentiated eell mass; dr, dorsal root; dut, deseending limb of uterus; eb, excretory bladder; ec, exeretory canal; ep, excretory pore; f, fecundarium; g, gonopore; i, intestine; lc, Laurer's eanal; o, ovary; oo, ootype; ph, pharynx; pt, posterior testis; sc, sense cells; ut, uterus; vd, vas deferens; ve, vas efferens; y, yolk glands; yr, yolk reservoir; yd, yolk duct.